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U.S. PATENT APPLICATION No. 09/940,941

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IN RE APPLN. OF: SOGABE ET AL.  
PATENT APPLICATION No. 09/940,941  
FILED: AUGUST 28, 2001  
FOR: CREATINE AMIDINOHYDROLASE, PRODUCTION  
THEREOF AND USE THEREOF

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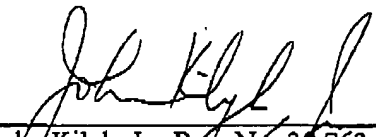
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Application No. 09/940,941

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In re Appln. of Sogabe et al.  
Application No. 09/940,941

relied upon for an earlier filing date under 35 USC 120 in which copies of the references were previously furnished are set out below:

U.S. APPLICATIONS		Status (check one)		
U.S. APPLICATIONS	U.S. FILING DATE	PATENTED	PENDING	ABANDONED
1.				
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**Partial English translation of JP 62-91182 A**

The physico-chemical characteristics of the creatine amidinohydrolase obtained in this invention is set forth in the following.

**① Action**

This enzyme hydrolyzes creatine to produce urea and sarcosine. The  $K_m$  (Michaelis constant) value for creatine is  $4.83 \times 10^{-3}$  M (37°C, pH 7.8).

**② Determination method of activity**

To 50 mM phosphate buffer (pH 7.8, 1.0 ml) containing 0.1 M creatine is added a properly diluted enzyme solution (0.1 ml), and the mixture is reacted at 37°C for 10 minutes. To the mixture is added a *p*-dimethyl aminobenzaldehyde solution (2.0 ml) (which is prepared by dissolving dimethyl aminobenzaldehyde (2.0 g) in dimethyl sulfoxide (100 ml) and adding conc. HCl (15 ml) thereto), and the mixture is stood at 25°C for 20 minutes. The absorbance ( $\Delta OD$ ) at 435 nm is determined with a spectrophotometer using the sample at 0 minute of the enzyme reaction as a reference, and the enzyme activity is calculated as follows.

Enzyme activity unit per 1 ml of the enzyme solution (U/ml)=

$$\Delta OD \times 9.66 \times \text{enzyme dilution fold}$$

wherein the enzyme activity of creatine amidinohydrolase is defined that the enzyme amount that produces 1  $\mu$ mol of urea per minute under the above-mentioned conditions is one unit (1U).

**③ Optimal pH**

The optimal pH is near 7.0-8.0.

The buffers used are phosphate buffers (pH 6.0-8.0), Tris-HCl buffers (pH 7.0-9.0), and carbonate buffers (pH 8.5-9.5).

**④ pH stability**

An enzyme was added to buffers having various pH values, the mixture was incubated at 5°C for 48 hours, and the residual enzyme activity was determined. The buffers used are the same as above. As a result, the creatine amidinohydrolase is found to be stable at near pH 7.0-8.5.

**⑤ Heat stability**

A solution (1.0 ml) of creatine amidinohydrolase in 50 mM phosphate buffer (pH 7.5) was treated at various temperatures for 30 minutes, and the residual activity of the enzyme was determined. As a result, the creatine amidinohydrolase is found to be stable at near 40°C or below.

**⑥ Optimal temperature**

The optimal temperature for the reaction of the creatine amidinohydrolase is found to be near 40°C.

**⑦ Molecular weight**

About 50,000 (determined by gel filtration)